DETERMINATION OF SPECIFIC MORINGA DIVERSITY IN THE PALU VALLEY BASED ON MORPHOLOGICAL AND GENETIC ANALYSIS

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(Received 26th Dec 2022; accepted 23rd Sep 2024)

Abstract. *Moringa oleifera* L. is a valuable commodity with various health benefits, especially during the COVID-19 pandemic. In developing and improving the potential of *Moringa oleifera* (MO) in the Palu Valley (Indonesia) to meet the need for quality seeds, a study of the diversity of morphology, seed viability, and genetics is needed. This study aims to identify MO accession based on morphological characteristics, viability, and genetic testing. Morphological analysis was carried out on 24 agronomic characters; a seed viability test was carried out using a Complete Randomized Design in selected accessions. The parallel genetic analysis was performed through DNA extraction using the PCR method using 10 ISSR primers. The data were analyzed using cluster analysis using the SYSTAT program in the form of a dendrogram. The study results obtained 24 accessions of MO with different morphological characters. Germination power ranged from 83.33 to 88.33 %, the speed of shoot emergence was 3.42 to 6.1 days, and the Euclidian distance was 0.290. There were five different MO groups of genotypes, each represented by accession Kulawi2, Kulawi10, Palolo24, Balaroa5, and Tondo19. It is suggested that five selected MO accession groups can be used as mother trees for seed supply.
Keywords: *cluster analysis, ISSR, mother tree, seed, viability*

Introduction

The MO plant originated in India but now widely spreads in the tropics, especially in Indonesia, both in the lowlands and the highlands, as an endemic plant (Popoola and Obembe, 2013; Bayé-Niwah and Mapongmetsem, 2014). The potential of MO as a "miracle" plant is undeniable (Ali Redha et al., 2021; Osman and El-Naggar, 2022; Yadav et al., 2024). Especially today, as 152 countries in the world have been struck by the COVID-19 pandemic, which has crippled various aspects of human life, such as social, economic, political, and cultural.

For generations, people in Indonesia have planted MO as a land barrier in rice fields or yards, as fences, or for reforestation without being identified (Simbolon et al., 2008; Afrianto and Metananda, 2024). It is used as a highly nutritious vegetable or animal feed; in certain tribes, it is even used as a repellent to evil spirits. Along those lines, different groups argue that Moringa leaves have been recognized for centuries as a versatile, nutrient-rich, and healing plant (Avilés-Gaxiola et al., 2021), including preventing coronavirus infections (Retolaza, 2020; Siddiqui et al., 2022; Yousefi Rad et al., 2024), HIV/AIDS (Kuete, 2017) and other diseases because they contain more diverse natural compounds than various types of plants.

Recent studies generally focus on the use of property and various derivative products. However, research and publications on plant cultivation and technology are still minimal. Proper cultivation will not produce quality raw materials; therefore, this study does not

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_55935616

discuss the chemical composition of leaves and stems. Substandard planting materials will hurt market and industrial demands. Consequently, it is necessary to improve its cultivation technology, including determining the mother tree as a source of quality Moringa seeds by testing the viability of seeds after morphological identification and determining their genotype groups.

From the description above, research on the diversity of MO species in the Palu Valley based on morphological analysis, seed viability, and genetics yield genotype group is strategic to improving MO cultivation technology and procuring quality seeds resilient to global climate change.

Review of literature

The morphological diversity of MO on six accessions of Bogor, Cirebon, Kediri, Pasuruan, Probolinggo, and Lhokseumawe (Indonesia) to the response of leaf production has been researched (Saputra et al., 2020), obtaining the highest wet weight results in the accession of Bogor and Cirebon (Indonesia). The level of MO kinship in Padang City, Padang Panjang Regency, Padang Pariaman City, and Agam Regency (Indonesia) has been examined on morphological differences to determine the mother tree analyzed using ArcGIS (Setiawan et al., 2020). In contrast, MO's qualitative and quantitative characteristics were analyzed by clusters using PBstat. The results showed that 29 MOs were found with different attributes in the research, which characterizes MO fruits and seeds in Salut Village, North Lombok Regency, Indonesia; the research data were analyzed descriptively, followed by cluster analysis using the SPSS 20 program (Jannah et al., 2020). The results showed that the fruit, winged seeds, and the 100 heaviest seeds varied from one hamlet to another but were known to be the closest kinship shown by the populations of Sambik Rindang 2 and Sambik Rindang 8 (2.44). In contrast, the farthest was demonstrated between the populations of Sambik Rindang 1 and Tanak Sebang 6 (67.87). Based on the results of MO morphological diversity studies that have been carried out, it appears that there has been no research that leads to testing the viability and vigor of seeds for the provision of quality seeds because an increase in yield cannot be achieved without starting with the provision of quality seeds that lead to the determination of the mother tree.

Several researchers have researched MO diversity and found the genetic stability of *Moringa peregrine* L. using CTAB and PCR extraction analysis methods in Saudi Arabia, as well as other studies focusing more on the purpose of treatment and health (Alaklabi, 2015). Using the RAPD marker method to measure DNA concentration and purification, the research explores the genetic diversity of four types of MO from East Flores Regency, Indonesia, and its connection to chemical composition and in vitro gas production; Kjeldhal and HPLC were used to analyze the composition of proximates and amino acids (AA), and IVGP was evaluated (Kleden et al., 2017). Four distinct MO morphologies were discovered: aromatic green, reddish-green, red, and green. Examining RAPD findings indicates a 68.8-74.7% genetic similarity, suggesting that the MO has significant genetic similarities. Morphological variations in leaves can also be linked to differences in their chemical composition, as observed in a study that examined genetic diversity in certain MOs. An investigation on landraces from Western Nigeria employing RAPD markers demonstrated that the abundance of polymorphism obtained supports the effectiveness of RAPD in studying genetic diversity in MO (Ojuederie et al., 2012). Moreover, the research conducted on Poteran Island-Madura (Indonesia) to study the genetic diversity of MO, explicitly focusing on Petiole Colors and utilizing the ISSR

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Method, reported a significant level of polymorphism. The ISSR marker generated a polymorphism rate of 97.2%, with an average PIC of 0.46 and a similarity value ranging from 5% to 66.7% (Muslihatin et al., 2022).

Analysis of MO genetic diversity has also been performed in Nigeria (Abubakar et al., 2011). Using RAPD primers 24 and 10, another researcher observed polymorphisms in 74% and 81.5% of cases (Ojuederie et al., 2012). They further examined the genetic relationships among 20 organisms from Malaysia by employing 24 RAPD primers, which yielded a polymorphism rate of 32.7%. Using molecular markers is indispensable in characterizing germplasm MO for assessing genetic diversity and establishing genetic relationships between different genotypes. DNA sequences that vary at specific locations in the genome are known as molecular markers and can be employed to distinguish individuals or species (Hendre Prasad and Aggarwal, 2007).

The reason for selecting inter simple sequence repeat (ISSR) molecular markers in this study was the abundance of microsatellite primers in eukaryotic organisms, specifically plants. Therefore, they achieved a higher amplification success rate and opted for more extended primers (16-25 bases) instead of RAPD primers (10 bases). Researchers have applied genetic diversity analysis using ISSR in *Murraya koenigii* (Verma and Rana, 2011), *Jatropha curcas* (Wasiu Arolu et al., 2011), *Punica granatum* (Parvaresh et al., 2012), *Brassica napus* (Abdelmigid, 2012), and MO in India (Saini et al., 2013). The genetic diversity of MO in India was examined using six primers, and the ISSR analysis revealed a 48.57% polymorphism rate (Saini et al., 2013). To determine the genetic distribution of different plant morphological characteristics, using molecular markers is the most effective approach (Ojuederie et al., 2012).

MO in Central Sulawesi, particularly in the Palu Valley, shows excellent morphological diversity, but seed viability analysis and genetic diversity studies have yet to be conducted. Thus, it is crucial to assess the viability of seeds and the diversity of genes by employing inter simple sequence repeat (ISSR) molecular markers. This analysis will help determine the closeness of the relationships depicted in the dendrogram. This step is considered strategic in obtaining the mother tree as a source of quality seeds for developing MO plantations as raw export materials.

Materials and methods

Description of the site and sample collection

The research was carried out in the villages of MO planting centers found in the Palu Valley (Indonesia), located in Tondo, Balaroa, Dolo, Palolo, and Kulawi, the Central Laboratory of Agrotechnology, Tadulako University, Palu, and the Biotechnology Laboratory of Brawijaya University Malang, in May – December 2020 and April – November 2022. This study uses a descriptive and direct survey method to determine the location chosen purposively, considering that the area has a more dominant MO plant population than other regions. It is also based on information from the Central Sulawesi Provincial Plantation Office. The sample was randomly selected; each village selected 30 trees, so 150 trees were used. The MO trees used as samples are moringa trees with the following criteria: tree age between 6 – 15 years, healthy leaf condition, sturdy trunk, branches, good growth, unpolluted growing environment, free from pests and diseases, and well maintained.

The tools used in this study include a description of the MO modification (Mohamad, 2013) consisting of 24 characters, namely: plant age, plant height, rod diameter, header

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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diameter, header shape, flower colour, branching shape, the origin of the seed, leaf shape, angle of leaves to branches, leaf length, leaf width, petiole length, pelvinus thickness, leaf tip shape, the basic shape of leaves, indentation of leaf margin, pod length size, pod shape, fruit tip shape, fresh pod colour, dry pod colour, number of seeds/pods.

The material used in the genetic analysis is a variety of MO leaves and trees that are six years old and have been produced at least three times. The number of plants observed was 150 MO trees (stems and leaves), styrofoam primary aquadest10 ISSR (ISSR1-10), Chisam solution (chloroform: isoamyl alcohol = 24: 1), cold isopropanol, 70% ethanol solution, and one-time TE solution.

The collection of research samples was carried out using experimental and descriptive methods. The activity was carried out in several villages in five sub-districts in the Palu Valley, Central Sulawesi, by identifying the character of the MO plant as a candidate for the mother tree as a source of seeds. The distribution of MO tree locations is shown in *Figure 1*, and the climate and coordinates of some MO plants are shown in *Table 1*.

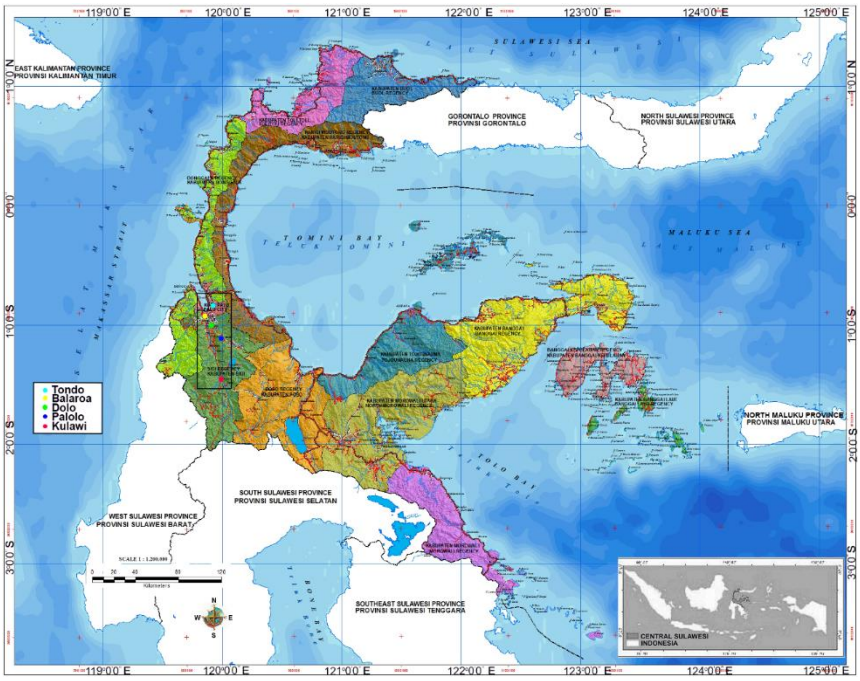

Figure 1. *Distribution of MO morphological identification sites in Palu Valley*

Morphological identification, viability test, and genetic identification

Morphological identification

The identified MO sample begins with visual morphological identification. The morphological 24-character data collected from 150 MO accessions is converted into binary data in matrix form. The similarity matrix between MO accession figures is calculated based on binary data. The similarity values are grouped into MO accession numbers using the Unweighted Pair Group Method with the Arithmetic (UPGMA) method. This grouping describes the relationship between the individual MO accessions observed based on the morphological character analyzed. The photo location of MO is illustrated in *Figure 2*. The results of morphological grouping were used for viability tests and genetic identification.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Table 1. *Coordinate climatological data in the MO planting area*

Regency	District	Village	Tree labels	South	East	Elevation (msl)	Temperature (°C) average	Humidity (%) average	Wind Velocity (Knots) average	Pressure of Atmosphere (Mb) Average	Amount of Precipitation (mm)	Amount of Rainy Days (day)	Duration of Sunshine (%)
Palu	Ulujadi	Donggala Kodi (Balaroa)	BL2	0° 54' 20.3"	119° 49' 54.6"	113	26.7 – 29.4	71.1 – 83.8	3 - 5	1010 - 1012	15 - 181	11 - 28	53 - 128
			BL4	0° 54' 22.7"	119° 50' 12.1"	79							
			BL5	0° 54' 22.8"	119° 50' 12.8"	78							
			BL7	0° 54' 31.1"	119° 50' 20.7"	54							
			BL9	0° 54' 27.9"	119° 50' 27.6"	43							
			BL10	0° 54' 16.0"	119° 50' 35.0"	31							
	Mantikulore	Tondo (Tondo)	TN14	0° 50' 43.9"	119° 54' 19.4"	136							
			TN15	0° 50' 44.1"	119° 54' 19.3"	137							
			TN18	0° 50' 48.5"	119° 54' 12.1"	124							
			TN19	0° 50' 39.9"	119° 54' 10.2"	127							
			TN20	0° 50' 39.8"	119° 54' 10.0"	127							
			TN 28	0° 50' 09.3"	119° 53' 20.7"	45							
Sigi	Palolo	Sigimpu (Palolo)	PL11	1° 04' 56.3"	119° 58' 27.2"	512	27.1 – 29.4	71.1 – 83.8	3 - 5	1009.8 – 1012.1	24 – 141.0	6 - 25	53 - 128
			PL21	1° 05' 13.3"	119° 58' 53.7"	581							
			PL24	1° 04' 57.2"	119° 58' 26.4"	518							
			PL28	1° 04' 52.8"	119° 58' 23.7"	517							
	Kulawi	Bolapapu, Namo (Kulawi)	KLW2	1° 26' 2.0"	119° 59' 14.7"	577							
			KLW4	1° 25' 58.8"	119° 59' 13.5"	576							
			KLW10	1° 25' 43.6"	119° 58' 59.3"	595							
			KLW15	1° 26' 8.8"	119° 59' 16.1"	581							
	Dolo	Kota Rindau, Kota Pulu (Dolo)	DL1	0° 58' 30.9"	119° 52' 43.8"	29							
			DL5	0° 58' 24.4"	119° 52' 58.6"	29							
			DL14	1° 00' 13.3"	119° 52' 35.4"	35							
			DL24	0° 58' 26.0"	119° 52' 52.1"	26							

Note: a) GPS Garmin GPSMAP 64sc measured the coordinates and elevation. b) climatological data issued in 2022 by the Central Bureau of Statistics Palu and Sigi Regency

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_55935616

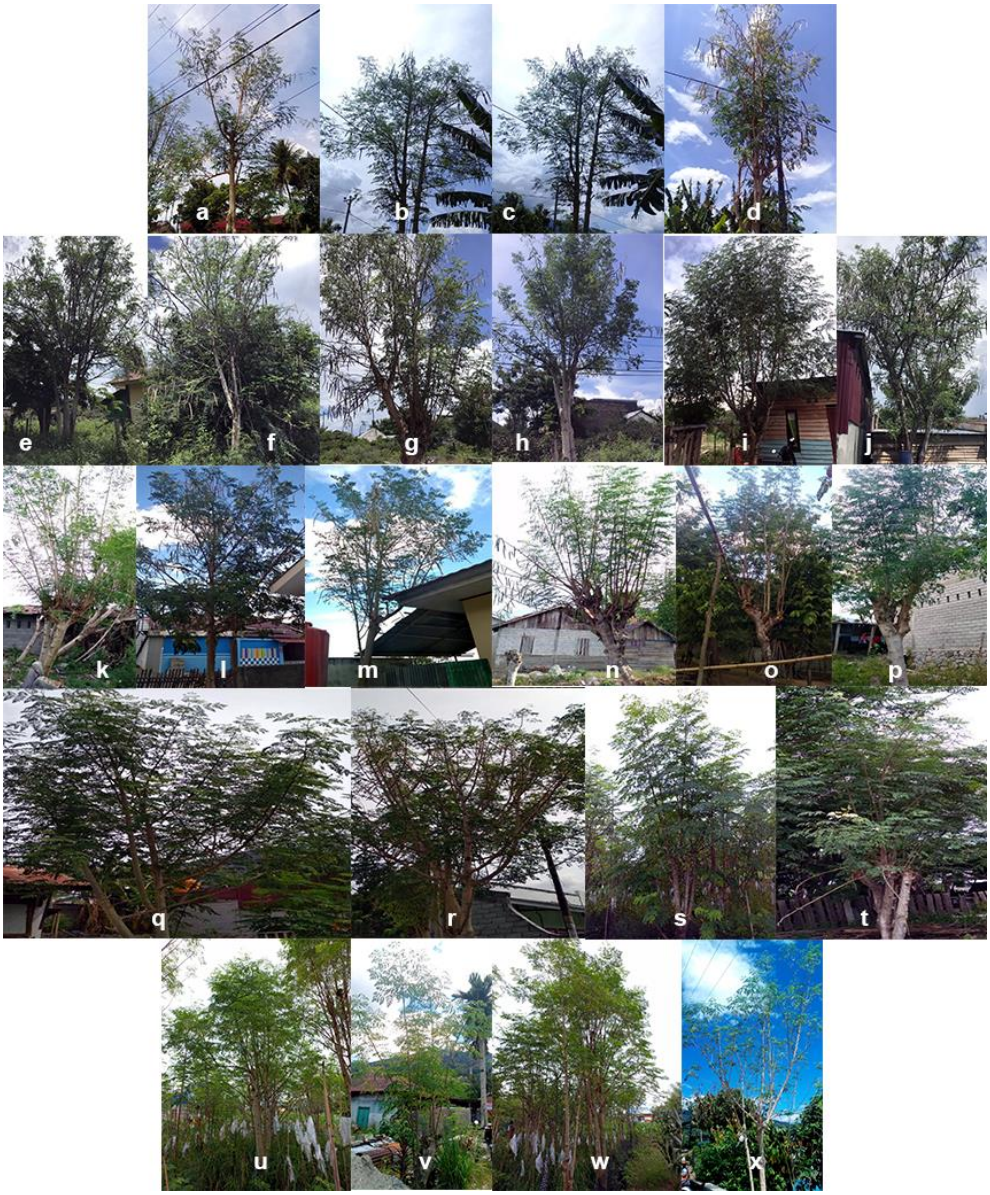

Figure 2. *Documentation of MO location: a) Dolo1, b) Dolo5, c) Dolo14, d) Dolo24, e) Tondo14, f) Tondo15, g) Tondo18, h) Tondo19, i) Tondo20, j) Tondo28, k) Balaroa2, l) Balaroa4, m) Balaroa5, n) Balaroa7, o) Balaroa9, p) Balaroa10, q) Kulawi2, r) Kulawi4, s) Kulawi10, t) Kulawi15, u) Palolo11, v) Palolo21, w) Palolo24, and x) Palolo28*

Viability test

Testing the viability of generative seeds using the one-factor Complete Random Design (CRD) method, namely MO accession consisting of 16 MO accessions: Dolo1-24, Balaroa2-10, and Tondo14-28; repeated three times, the total treatment was 48 units. Each unit uses 150 seeds: 50 grains for germination power testing, 50 for maximum growth potential testing, and 50 for germination speed. The total number of seeds used is 7200 seeds. Testing the viability of vegetative seeds using the one-factor Group Random Design (GRD) method, namely MO accession consisting of 8 MO accessions: Palolo11-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_55935616

28, Kulawi2-15; repeated four times so that the total treatment was 32 units. Each unit uses ten cuttings, so the number of cuttings used is 320.

The viability test of seeds on genetically different accessions was carried out using the ISTA Rules 2020 method of testing generative and vegetative seeds. Testing of seeds of vegetative origin was carried out on MO with pods containing tiny seeds and a small number. In seeds of generative origin, the Sand test was used with observation variables consisting of germination power, maximum growth potential, and growth speed. Testing of seeds of vegetative origin was carried out by planting seeds in sterile planting media, with the composition of soil, sand, and manure that is balanced and well drained. The observation variables were the Speed of Shoot Emergence, the Number of Shoots, the Length of Shoots, and the Number of Leaves Growing. The results of the seed viability test will be a recommendation for determining MO mother trees as a source of quality seeds. The viability test samples used were seeds, namely the Dolo, Balaroa, and Tondo genotypes from 26-137 msl, and MO cuttings, namely the Palolo and Kulawi genotypes from 512-595 msl.

Genetic identification

Genetic Identification uses molecular analysis by isolating DNA on fresh young leaves that have been taken, weighed as much as 0.1 grams, and then scraped using mortal and pistil with the help of liquid nitrogen. The following molecular analysis is DNA amplification using the ISSR molecular marking method with a Polymerase Chain Repeats (PCR) machine. The DNA isolation procedure uses the isolation kit Wizard Genomic DNA Purification Kit (Promega).

Mark ISSR has high codominant and polymorphism properties even in genotypes with a close degree of kinship. This study used ten specific primers (ISSR) (Shahzad et al., 2013). After amplification, the results of the PCR process will be electrophoresis using agarose gel and EtBr by Bio-Rad Gel Doc XR+ imaging system so that data can be analyzed and obtained in the form of matrices and dendrograms showing different genetic accession groups.

Data analysis

MO through morphological identification in 150 MO accessions consists of 24 observed morphological characters; the data obtained are converted into binary data in matrix form. The similarity values are grouped into MO accession numbers using the Unweighted Pair Group Method with the Arithmetic (UPGMA) method. The collected data were analyzed by calculating the distance Euclid connected based on the closest relationship using the SYSTAT program computer application. The cluster analysis results form the basis for selecting diverse MO accession groups for viability testing and genetic identification.

Cluster analysis is a method used in data analysis to group individuals or objects based on their shared characteristics. The goal is to create groups where the individuals or entities within each group have similar or identical characteristics. Morphological characters are easy to see, so their variations can be assessed quickly compared to others (Acito, 2023).

Cluster analysis analyses diversity and classifies plants based on morphologically identified data and parameters. Morphological characters are essential in systematics; although other approaches are commonly used in structuring classification systems, they all derive from morphological characters (Davis and Heywood, 1963).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Seed viability test data were analyzed using ANOVA, and significant differences in germination variables were determined using the Fisher difference test. If the treatment significantly affects it, it will continue with the 5% Honestly Significant Difference (HSD) test (Gomez and Gomez, 1976).

Results and discussion

Results

Morphological identification

Of the 150 accessions observed, 24 groups with morphological similarities were obtained. MO diversity through morphological identification in Dolo District (Dolo), Mantikulore District (Tondo), Ulujadi District (Balaroa), Palolo District (Palolo), and Kulawi District (Kulawi) is shown in *Figure 3*, and the measured parameters for the chosen MO are summarised in *Table 2*.

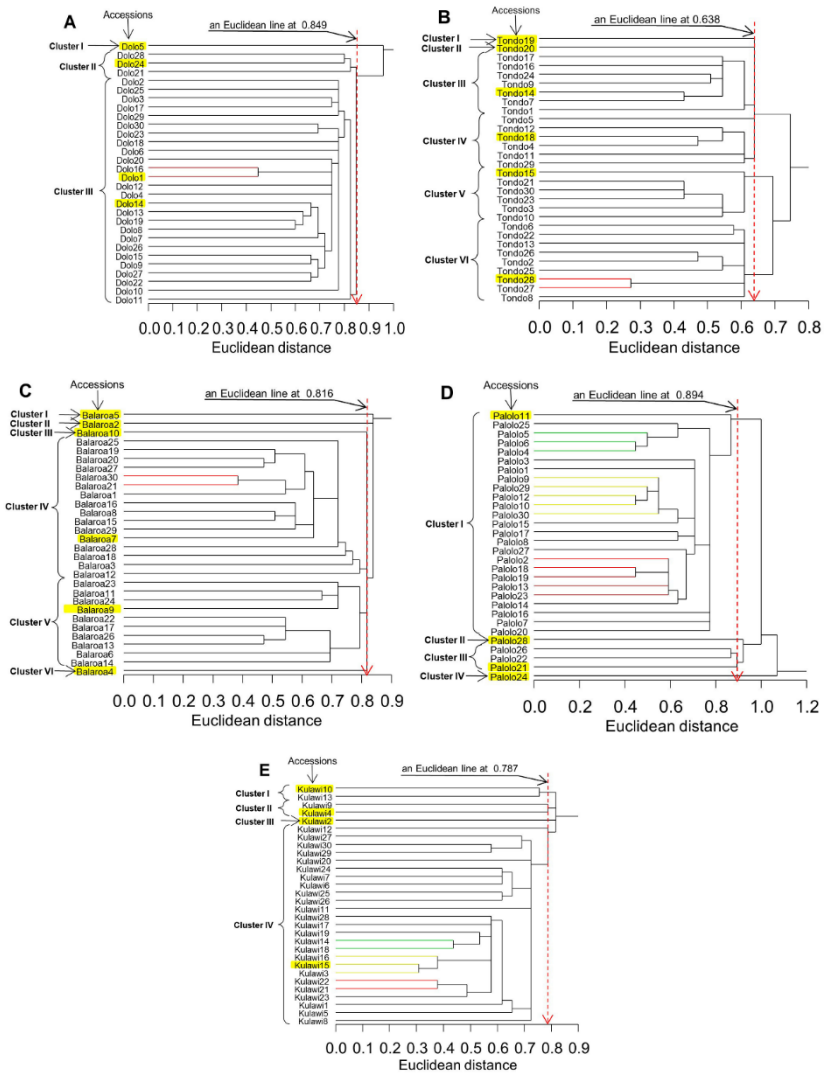

Figure 3. *Dendrogram grouping of MO based on morphological identification: (A) Dolo District (Dolo), (B) Mantikulore District (Tondo), (C) Ulujadi District (Balaroa), (D) Palolo District (Palolo), and (E) Kulawi District (Kulawi)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Table 2. *Morphological parameters of the chosen MO*

Characters	MORPHOLOGY																		
	Palolo11	Palolo21	Palolo24	Palolo28	Kulawi2	Kulawi4	Kulawi10	Kulawi15	Balaroa2	Balaroa4	Balaroa5	Balaroa7	Balaroa9	Balaroa10	Tondo14	Tondo18	Tondo19	Tondo20	Tondo28
PA (year)	6	6	6	6	8	12	6	14	6	6	6	6	6	6	6	6	6	6	6
PH (m)	5.5	6.5	6.1	5.7	5	4.3	7.3	4.5	11.3	13.2	10.5	9.3	9.4	11.1	10	10.2	9.2	8.2	10.1
RD (cm)	25	32	26	27	20	25	23	18	28	38	26	20	22	23	20	26	47	22	40
HD (m)	2	3.25	2.06	2.7	2.7	2.28	2.1	1.95	3.4	6.6	4.9	2.5	2.8	3.3	3.4	4.3	3.5	2.5	7
HS	irregular	fastigiate	fastigiate	fastigiate	irregular	irregular	oval	fastigiate	irregular	fastigiate	oval	vase	fastigiate	irregular	fastigiate	vase	fastigiate	fastigiate	irregular
SC	greyish beige	greyish beige	dirty white	greyish beige	dirty white	greyish beige	greyish beige	white bones	dirty white	dirty white	greyish beige	dirty white	white bones	greyish beige	greyish beige	white bones	greyish beige	white bones	dirty white
FC	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	with purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	without purple stripes	with purple stripes
BS	drooping branches	leaning upwards	upright	round	leaning upwards	leaning upwards	leaning upwards	leaning upwards	upright	upright	flat	upright	leaning upwards	flat	upright	upright	flat	flat	upright
TOS	planted	planted	planted	planted	planted	planted	planted	planted	grows on its own	grows on its own	planted	planted	planted	grows on its own	planted	planted	planted	planted	planted
LS	ovate	ovate	elliptical	lancet	oval	elliptical	inverted lancet	oval	inverted lancet	elliptical	inverted lancet	oval	lancet	inverted lancet	oval	oval	oval	oval	oval
ALB	faced	faced	faced	faced	faced	faced	faced	faced	faced	faced	faced	faced	faced	faced	reef	faced	faced	faced	faced
LL (cm)	10	7	8	13	13	20	21	20	19	27	22	10	22	23	19	17	16	19	17
LW (cm)	7	4	8	6	8	11	10	10	12	19	10	8	12	8	11	7	14	9	12
PL (cm)	50	45	35	30	25	45	45	39	39	38	44	39	39	39	45	40	35	35	35
PT	thin	thin	thin	thin	thin	thin	thick	thin	thin	thin	thin	thick	thin	thin	thick	thin	thin	thin	thin
LTS	pointed	rounded corners	blunt	pointed	rounded corners	blunt	rounded corners	rounded corners	pointed	rounded corners	blunt	rounded corners	blunt	rounded corners	rounded corners	blunt	rounded corners	rounded corners	rounded corners
BSL	pointed	pointed	pointed	pointed	blunt	rounded corners	rounded corners	rounded corners	blunt	pointed	pointed	rounded corners	blunt	blunt	blunt	pointed	rounded corners	rounded corners	blunt
ILM	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat	flat
PLS (cm)									50	51	40	35	45	35	33	38	42	32	37

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Characters	MORPHOLOGY																		
	Palolo11	Palolo21	Palolo24	Palolo28	Kulawi2	Kulawi4	Kulawi10	Kulawi15	Balaroa2	Balaroa4	Balaroa5	Balaroa7	Balaroa9	Balaroa10	Tondo14	Tondo18	Tondo19	Tondo20	Tondo28
PS									straight	straight	curvy	straight	curvy	straight	straight	straight	straight	straight	straight
FTS									taper	taper	taper	taper	taper	taper	taper	taper	taper	taper	taper
FPC									dark green	dark green	dark green	dark green	dark green	dark green	dark green	dark green	dark green	dark green	dark green
DPC									dark brown	dark brown	greyish brown	dark brown	dark brown	greyish brown	dark brown	dark brown	dark brown	dark brown	dark brown
NOSP									25	25	20	30	20	25	25	20	25	30	20

Notes: plant age (PA), plant height (PH), rod diameter (RD), header diameter (HD), header shape (HS), stem colour (SC), flower color (FC), branching shape (BS), the origin of seed (TOS), leaf shape (LS), angle of leaves to branches (ALB), leaf length (LL), leaf width (LW), petiole length (PL), pelvinus thickness (PT), leaf tip shape (LTS), basic shape of leaves (BSL), indentation or leaf margin (ILM), pod length size (PLS), pod shape (PS), fruit tip shape (FTS), fresh pod color (FPC), dry pod color (DPC), number of seeds/pods (NOSP)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Figure 3 shows five different morphological groups of MO Dolo District (Dolo) at a distance of 0.849 Euclidean, represented by the Dolo14, Dolo24, Dolo5, and Dolo1 accessions. In Mantikulore District, at a distance of 0.638, Euclidean is defined by the Tondo14, Tondo15, Tondo18, Tondo19, Tondo20, and Tondo28 accessions. In Ulujadi District (Balaroa), at a distance of 0.816, Euclidean is represented by the accessions Balaroa2, Balaroa4, Balaroa5, Balaroa7, Balaroa9, and Balaroa10. Palolo District (Palolo), at a distance of 0.894 Euclidean, is represented by the Palolo11, Palolo21, Palolo24, and Palolo28 accessions. In Kulawi District (Kulawi), at a distance of 0.787, Euclidean is defined by the accessions of Kulawi2, Kulawi4, Kulawi10, and Kulawi15.

The results of the morphological identification of MO showed that the morphological characters are chosen generally showed diversity, such as stem colour: dirty white, white bones, and greyish beige; leaf shape: oval, elliptical, inverted lancet, ovate, and lancet; leaf width measuring 4 to 19 cm; petiole length 25 to 50 cm; leaf length 7 to 27 cm; and pod length size 32 to 51 cm.

Differences in morphological characteristics are possible because the moringa observed is at different altitudes, namely 20 to 600 meters above sea level; on the other hand, MO plants carry out cross-pollination more dominantly, although they can pollinate themselves; this provides an opportunity for variation other than due to genetic characteristics.

Seed viability test

a, Generative (seed)

Figure 4 shows the following results based on the viability and vigor tests of MO seeds in seedlings and nurseries.

Based on *Figure 4* shows that all MO accessions tested had a percentage of germination not significantly different, ranging from 83.33% to 88.33%. Tondo14, Tondo18, and Tondo28 accessions tend to be higher than others. All MO accessions tested had a maximum growth potential percentage that did not differ markedly, ranging from 95% to 100%. Tondo14, Tondo18, Tondo28, Balaroa4, and Balaroa7 accessions are higher than others. All MO accessions tested had no different growing speed; the Balaroa4 accession showed that the growing speed tended to be faster at 28.65%/etmal.

b, Vegetative (cuttings)

The speed of shoot emergence

The results of the variety analysis on the speed of shoot emergence variable showed that neither Palolo nor Kulawi accession had any effect on the variable of the speed of shoot emergence. In *Figure 5*, the Palolo11 accession tends to have the fastest shoot emergence speed compared to other accessions, which is 3.42 days.

Number of shoots, length of shoots, and number of leaves growing

Based on the variety analysis, it was shown that Palolo's accession did not affect the variable of length of shoots formed at 2 WAP and 8 WAP, but at 4 WAP and 6 WAP, it showed a difference (*Table 3*). At 4 WAP, Palolo21 and Palolo11 accessions showed the best length of shoots, which was 1.5 cm, and was no different from Palolo24, but these three accessions differed from Palolo28. At 6 WAP, the Palolo21 accession showed the best length of shoot at 2.24 cm, which differed from the other three. The Kulawi accession showed that the observations of 2 WAP, 4 WAP, and 8 WAP did not affect the variable length of shoots. However, at 6 WAP, the Kulawi15 accession produced the best length of shoots of 1.74 cm and differed from the other three accessions.

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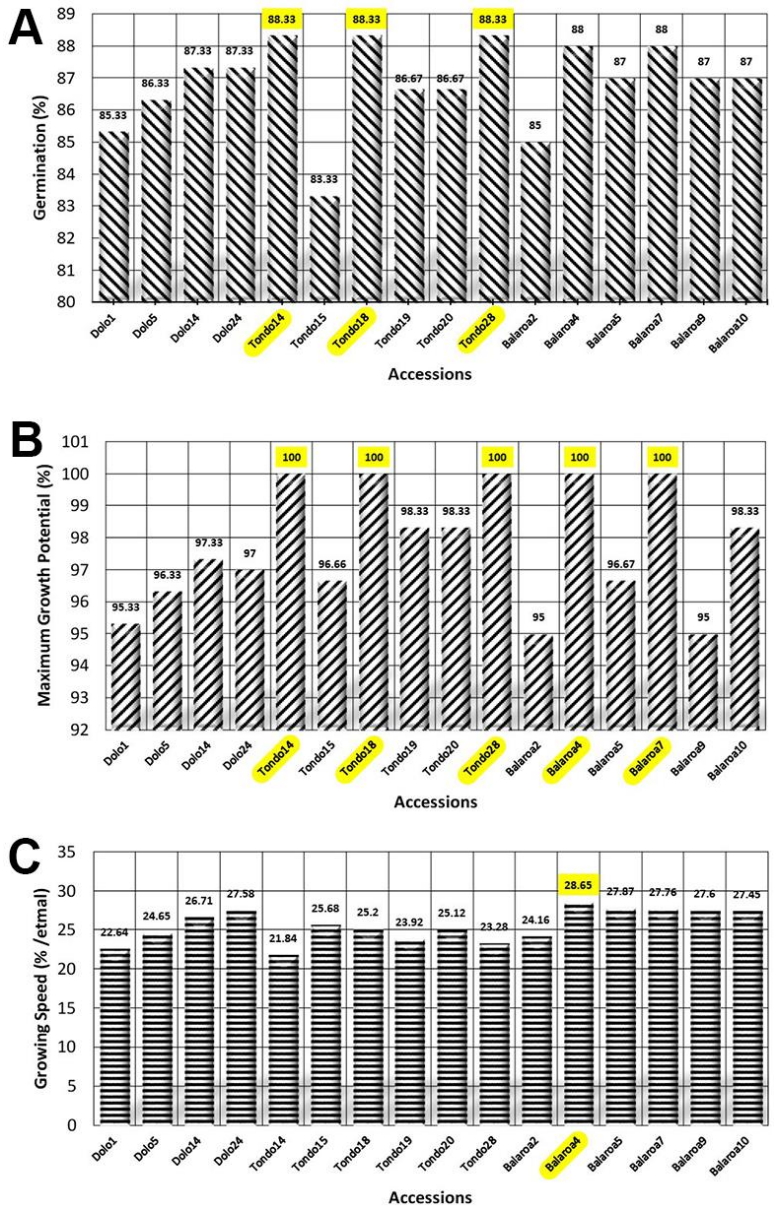

Figure 4. *Germination(A), maximum growth potential(B), and growing speed(C) of MO morphological identification sites in Palu Valley*

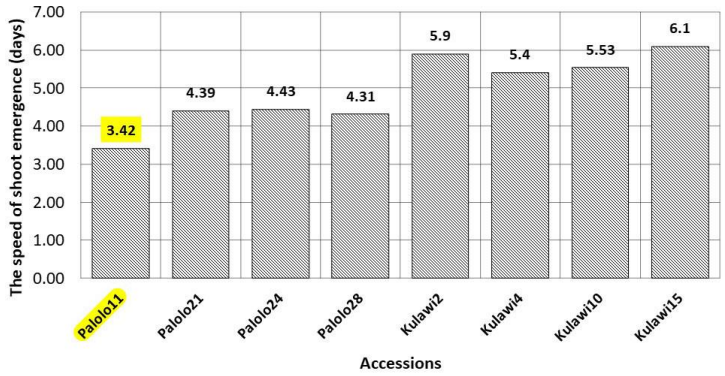

Figure 5. *The speed at which MO shoots appears on different accessions*

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Table 3. *Average of the length and number of shoots MO accession of Palolo and Kulawi*

Treatment	length of shoots (cm)		number of shoots (pieces)
	4 WAP	6 WAP	6 WAP
Palolo11	1.50[b]	1.85[b]	1.88[b]
Palolo21	1.50[b]	2.24[c]	1.92[b]
Palolo24	1.27[b]	1.49[a]	1.73[ab]
Palolo28	0.93[a]	1.34[a]	1.55[a]
HSD 5%	0.25	0.21	0.21
Kulawi2		1.52[a]	1.48[b]
Kulawi4		1.47[a]	1.28[a]
Kulawi10		1.48[a]	1.43[ab]
Kulawi15		1.74[b]	1.58[b]
HSD 5%		0.11	0.20

Note: The numbers in the column followed by the same letter do not differ markedly at HSD 5%

Variety analysis on the number of shoots showed that both Palolo and Kulawi accessions at 2 WAP, 4 WAP, and 8 WAP did not have an effect, except for the observation of 6 WAP, namely Palolo21 accession showed the highest number of shoots, which was 1.92 pieces and was no different from Palolo11 and Palolo24 accession but different from Palolo28. The Kulawi accession shows that the Kulawi15 accession has the highest number of shoots, 1.58 pieces. This is no different from the accession of Kulawi2 and Kulawi10, but it differs from Kulawi4.

Figure 6 shows the observation results for the number of shoots, the length of the shoots, and the number of leaves growing (which have no effect if analyzed statistically). Based on the figure, the increase of each variable in each accession shows a relatively similar trend. However, there seems to be a tendency for Palolo accession to produce higher values than Kulawi accession, especially in terms of the length of shoots and the number of leaves growing.

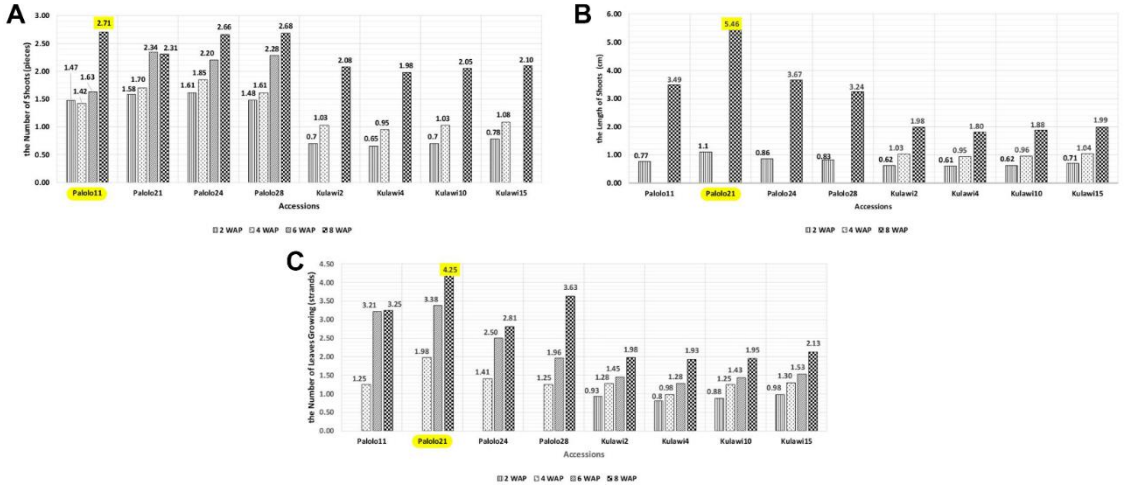

Figure 6. *The number of shoots(A), the length of shoots(B), and the number of leaves growing(C) on different MO accessions of Palolo and Kulawi*

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The results of the variety analysis on the number of leaves growing variable showed that neither Palolo nor Kulawi accession had any effect. In *Figure 6C*, the Palolo21 accession at 8 WAP tends to have the most leaves growing compared to other accessions, which is 4.25 strands.

Genetic identification

The diverse MO accession group derived from the results of morphological analysis is used for viability testing and genetic identification testing to obtain a diverse MO genotype that can be used for various purposes.

Based on genetic identification through DNA extraction and cluster analysis of 24 MO accessions (morphological analysis results) at a distance of Euclidean 0.290, 5 groups of MO plants with different genetic characters were obtained, each group represented by the accession of Kulawi2, Kulawi10, Palolo24, Balaroa5, and Tondo19, as shown in *Figure 7* and *Figure 8*.

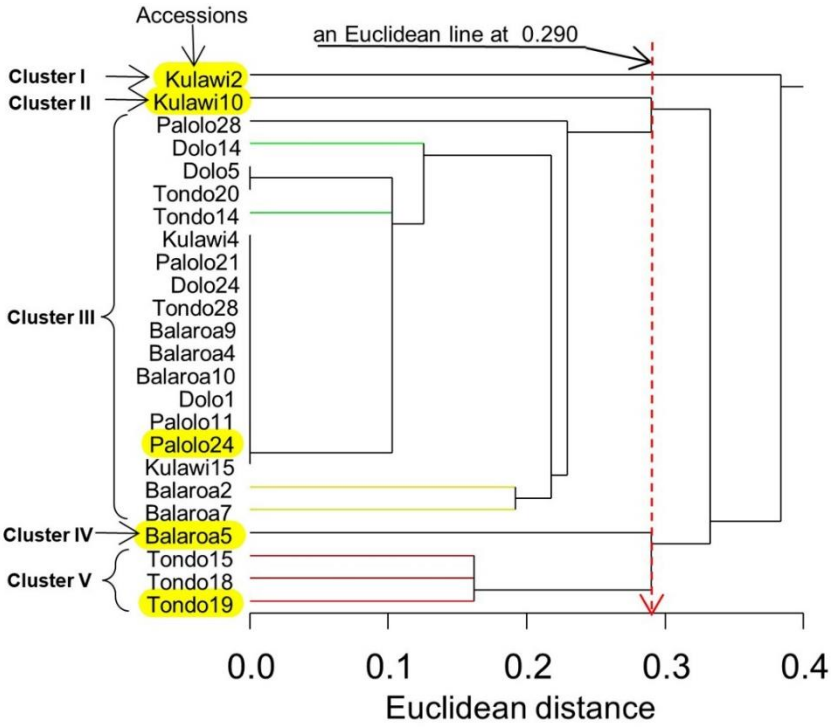

Figure 7. *Dendrogram grouping of MO based on genetic identification*

The Kulawi2 MO accession represents Cluster I; the Kulawi10 MO accession represents Cluster II; Cluster III is represented by the Palolo24 MO accession consisting of 17 accessions, namely Kulawi4, Kulawi15, Palolo28, Palolo21, Palolo11, Dolo11, Dolo14, Dolo5, Dolo24, Dolo1, Tondo20, Tondo14, Tondo28, Balaroa9, Balaroa4, Balaroa10, Balaroa2, and Balaroa7; the Balaroa5 MO accession represents Cluster IV, and cluster V is represented by the Tondo19 MO accession, which consists of two accessions: Tondo18 and Tondo15.

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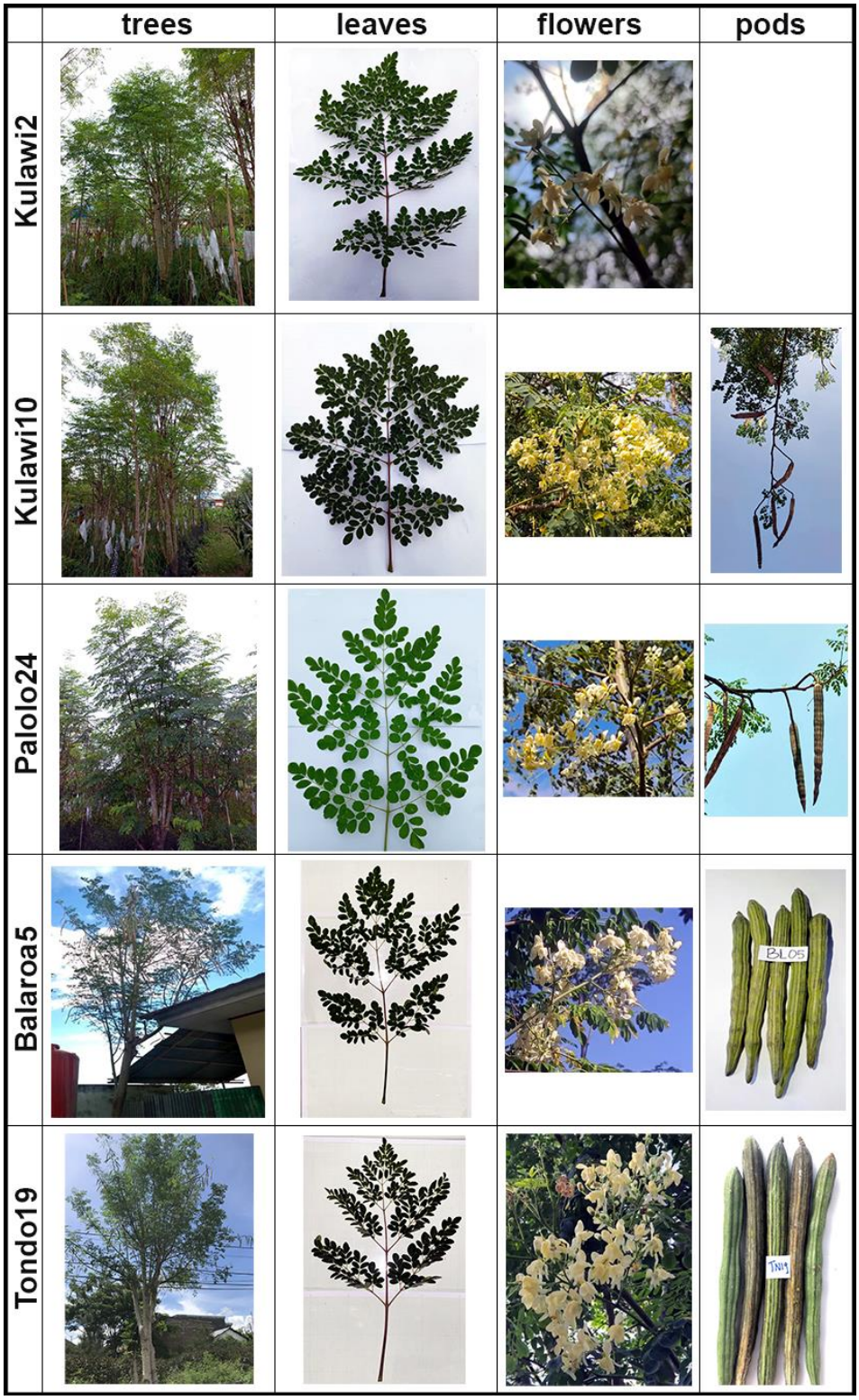

Figure 8. *Documentation of the chosen MO by genetics*

Furthermore, 24 MO accessions were identified by their genetic characters through DNA amplification using ISSR molecular markers. The results of DNA amplification are shown in *Table 4*.

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Table 4. *DNA Amplification Results Using ISSR Molecular Markers*

No	Primer	5' - 3'	Ribbon size	Ribbon				Total
				monomorfic		polimorfic		
1	$(GA)_8YG$	GAG AGA GAG AGA GAG ACT G	400 - 900	2	33.33%	4	66.67%	6
2	$(CT)_8RG$	CTC TCT CTC TCT CTC TAG G						
3	$(CA)_5RY$	CAC ACA CAC AAG CT	700 - 1800	2	40.00%	3	60.00%	5
4	$(CA)_5RG$	CAC ACA CAC AAG G	300 - 1800	3	42.86%	4	57.14%	7
5	$RY(GACA)_4$	AGC TGA CAG ACA GAC AGA CA	400 - 2100		0.00%	12	100.00%	12
6	$(ATG)_6$	ATG ATG ATG ATG ATG ATG	600 - 2500		0.00%	9	100.00%	9
7	$(GA)_8A$	GAG AGA GAG AGA GAG AA	500 - 1400	2	18.18%	9	81.82%	11
8	$(GA)_8YC$	GAG AGA GAG AGA GAG ACT C	500	1	100.00%		0.00%	1
9	$VHV(GT)_7$	ACG ACT ACG GTG TGT GTG TGT GT	300 - 2100	2	18.18%	9	81.82%	11
10	$HVH(TG)_7$	ACT ACG ACT TGT GTG TGT GTG TG	600 - 2000		0.00%	7	100.00%	7
				12	17.39%	57	82.61%	69

When using 10 ISSR primers, the amplification of MO DNA results in a range of 5 to 12 bands per sample per primer. There were 69 bands, with amplified DNA fragments ranging from 300 to 2500 bp. Sixty-nine bands were produced using 10 ISSR primers; 57 (82.61%) polymorphic bands and 12 (17.39%) monomorphic bands were obtained. Three of the ten primers used showed high levels of polymorphism (100%), namely RY $(GACA)_4$, $(ATG)_6$, and $HVH(TG)_7$ primers. In contrast, the primer produces at least one band (1.45%), i.e., $(GA)_8YC$. Primer has the most out of 12 bands (17.39%), namely $RY(GACA)_4$.

The DNA amplification results with 10 ISSR primers indicate variations in the number and intensity of DNA bands produced by each primer (refer to *Figure 9* and *Figure 10*). The primary sequence and genetic variations influence the detection of DNA bands with each primer (Tingey et al., 1994). The percentage of identical accession by DNA band for each primer varied: 37.50% $(GA)_8YG$, 20.83% $(CA)_5RY$, 0% $(CA)_5RG$, 0% $RY(GACA)_4$, 29.17% $(ATG)_6$, 20.83% $(GA)_8A$, 25% $(GA)_8YC$, 12.50% $VHV(GT)_7$ and 0% $HVH(TG)_7$, respectively. Based on all primary, the percentage of identical accession is 14.58%, and the proportion of genetically varying accessions is 75.42% (*Table 5*).

A display of DNA extraction results using 10 ISSR primers is shown in *Figure 9* and *Figure 10*.

Table 5. *MO accession percentages are identical and non-identical based on DNA bands*

No	Primer	5' - 3'	Ribbon size	identical		unidentical		Total
1	$(GA)_8YG$	GAG AGA GAG AGA GAG ACT G	400 - 900	9	37.50%	15	62.50%	24
2	$(CT)_8RG$	CTC TCT CTC TCT CTC TAG G						24
3	$(CA)_5RY$	CAC ACA CAC AAG CT	700 - 1800	5	20.83%	19	79.17%	24
4	$(CA)_5RG$	CAC ACA CAC AAG G	300 - 1800		0.00%	24	100.00%	24
5	$RY(GACA)_4$	AGC TGA CAG ACA GAC AGA CA	400 - 2100		0.00%	24	100.00%	24
6	$(ATG)_6$	ATG ATG ATG ATG ATG ATG	600 - 2500	7	29.17%	17	70.83%	24
7	$(GA)_8A$	GAG AGA GAG AGA GAG AA	500 - 1400	5	20.83%	19	79.17%	24
8	$(GA)_8YC$	GAG AGA GAG AGA GAG ACT C	500	6	25.00%	18	75.00%	24
9	$VHV(GT)_7$	ACG ACT ACG GTG TGT GTG TGT GT	300 - 2100	3	12.50%	21	87.50%	24
10	$HVH(TG)_7$	ACT ACG ACT TGT GTG TGT GTG TG	600 - 2000		0.00%	24	100.00%	24
				35	14.58%	181	75.42%	240

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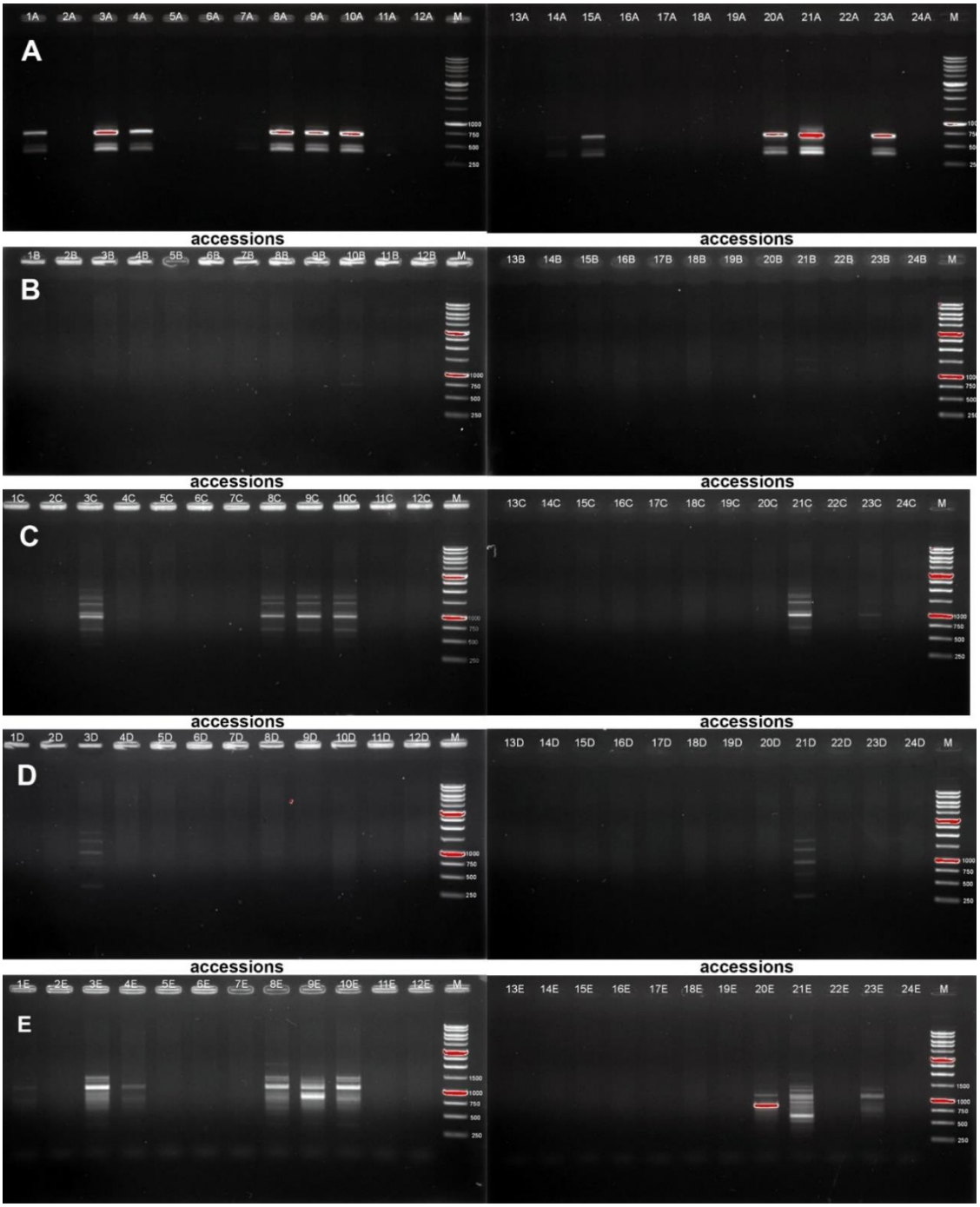

Figure 9. *MO accession DNA amplification using ISSR primers: primer 1(A), primer 2(B), primer 3(C), primer 4(D), and primer 5(E); accession 1(Balaroa2), accession 2 (Balaroa4), accession 3(Balaroa5), accession 4(Balaroa7), accession 5(Balaroa9), accession 6(Balaroa10), accession 7(Tondo14), accession 8(Tondo15), accession 9 (Tondo18), accession 10(Tondo19), accession 11(Tondo20), accession 12 (Tondo28), accession 13(Dolo1), accession 14(Dolo5), accession 15(Dolo14), accession 16 (Dolo24), accession 17(Palolo11), accession 18(Palolo21), accession 19(Palolo24), accession 20(Palolo28), accession 21(Kulawi2), accession 22(Kulawi4), accession 23 (Kulawi10), and accession 24(Kulawi15)*

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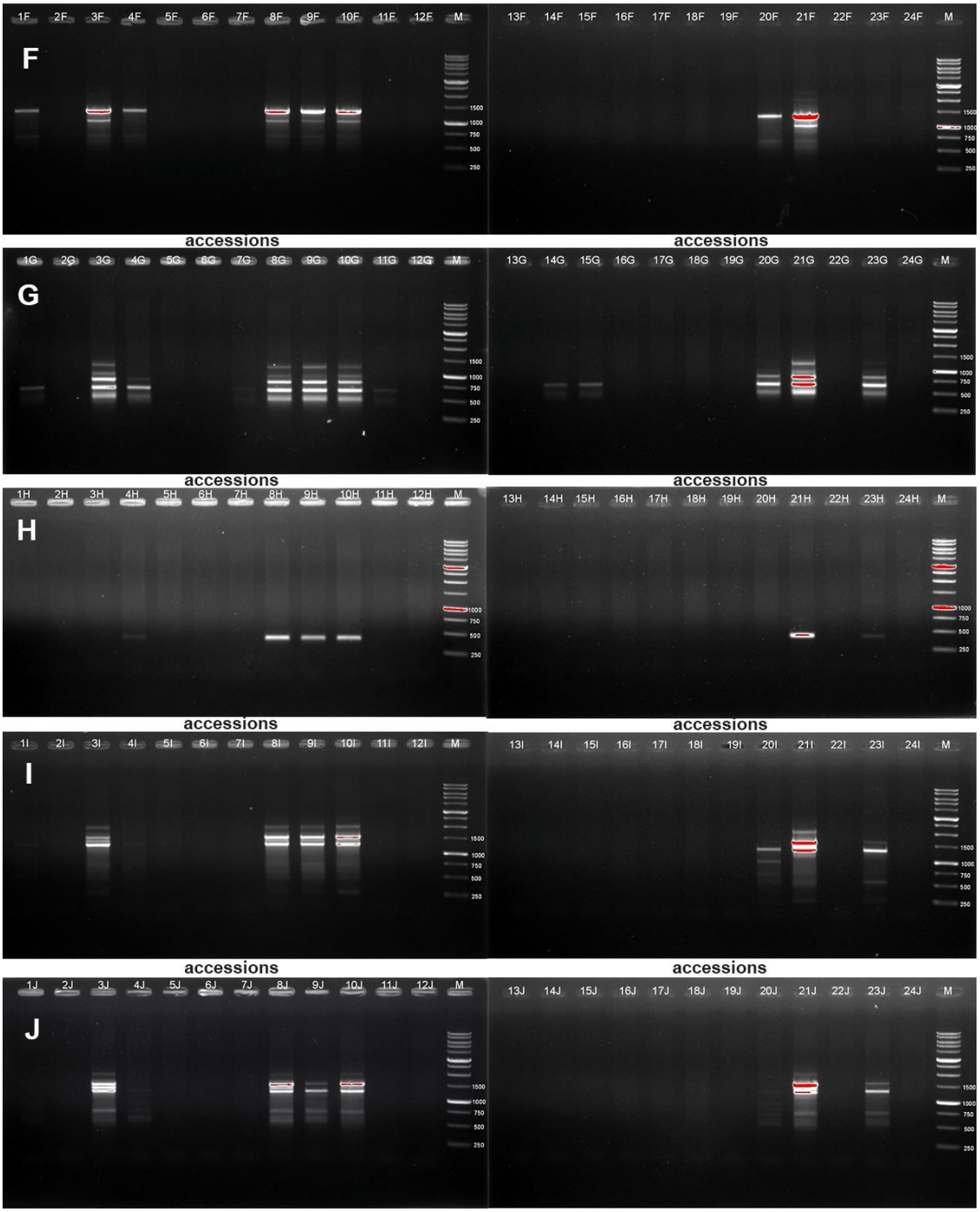

Figure 10. *MO accession DNA amplification using ISSR primers: primer 6(F), primer 7(G), primer 8(H), primer 9(I), and primer 10(J); accession 1(Balaroa2), accession 2 (Balaroa4), accession 3(Balaroa5), accession 4(Balaroa7), accession 5(Balaroa9), accession 6(Balaroa10), accession 7(Tondo14), accession 8(Tondo15), accession 9 (Tondo18), accession 10(Tondo19), accession 11(Tondo20), accession 12 (Tondo28), accession 13(Dolo1), accession 14(Dolo5), accession 15(Dolo14), accession 16 (Dolo24), accession 17(Palolo11), accession 18(Palolo21), accession 19(Palolo24), accession 20(Palolo28), accession 21(Kulawi2), accession 22(Kulawi4), accession 23 (Kulawi10), and accession 24(Kulawi15)*

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Discussion

The morphological diversity of 150 MO trees of Palu Valley is distinguished by the character: plant age, plant height, rod diameter, header diameter, header shape, flower colour, branching shape, the origin of the seed, leaf shape, angle of leaves to branches, leaf length, leaf width, petiole length, pelvinus thickness, leaf tip shape, the basic shape of leaves, indentation of leaf margin, pod length size, pod shape, fruit tip shape, fresh pod colour, dry pod colour, number of seeds/pods. The cluster analysis results obtained 24 diverse accessions whose accession distribution was four trees in Dolo District, six in Mantikulore District, six in Ulujadi District, four in Palolo District, and four in Kulawi District. Different growing environments can cause diversity to occur. Understanding morphological diversity is crucial as it significantly assesses the genetic diversity among different MO accessions. Moreover, it aids in identifying the desired traits for future MO improvement programs (Alavilli et al., 2022). Growing environmental factors result in the pressure of natural selection from generation to generation so that populations with various local genotypes are adaptive, both quantitative and qualitative. The interaction of genotype and environment in each location produces the best phenotype from each location-specific genotype as a form of plant adaptation to a specific environment. The height of the growing place dramatically affects the daily temperature. Lower places get a higher intensity of sunlight. The place's elevation is a determining factor in the plant's growth (Laily et al., 2012), including secondary metabolites of MO (Juswardi et al., 2023) and an increased amount of Fe content at lower altitudes (Hamzah and Yusuf, 2019). The intensity of sunlight, air humidity, and soil conditions such as texture, moisture, nutrients, and soil pH influence it. The concept of epigenetics explains how organisms with the same genetic information can display different phenotypes in response to the environment in which they live. Epigenetics concerns molecular mechanisms such as the expression of specific genes, so exploring and testing the genes responsible for epigenetics is necessary.

Based on the results of viability and vigor testing of MO accession seeds Palolo, Tondo, Dolo, Kulawi, Balaroa, and Kulawi both through generative and vegetative viability tests, all accessions showed high viability and vigor values; namely, germination reaching a range of 83.33% to 88.33%, the maximum growth potential of 95% to 100%, the growth speed of 21.84 to 28.65%/etmal, this shows that the MO accessions tested have seed viability that meets the standards to be used as a seed source, namely germination power of 85% and maximum growth potential reaches 100% (National Standardization Agency, 1995). Germination power is a potential viability benchmark, which is a simulation of the ability of seeds to grow and typically produce under optimum conditions; maximum sprouting frequency is the percentage of the number of normal and abnormal sprouts of all seeds planted, and the speed of seed growth is a rapid seed reactivation process if the surrounding conditions are optimal and metabolic processes are not hampered (Sadjad, 1993). Likewise, with the viability test using vegetative organs in the accession of Palolo and Kulawi MO, it appears that the speed of shoot emergence is 3.42 to 6.1 days, the number of shoots is 2.10 to 2.71 pieces, the length of the shoots is 1.49 cm to 5.46 cm and the number of leaves growing is 2.13 to 4.25 strands, this shows relatively similar or no different results since the observation of 1 WAP to 8 WAP, apart from the variable length of shoots of Kulawi accession at 6 WAP and Palolo accession at 4 to 6 WAP, there is also variation in the number of shoots for Kulawi and Palolo accession at 6 WAP. The emergence of these differences is because the growth process of each accession is not the same, and each accession's genotype and growth environment

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are different. However, until the end of the observation (8 WAP), all the parameters of viability and vigor accession Palolo and Kulawi showed relatively the same results. Vigor is a healthy seed condition; if planted immediately, it germinates quickly, simultaneously, and uniformly in different environments and then experiences rapid growth under typical conditions in the field; this shows that the tested MO accessions have the viability of seeds that meet the standards to be used as a seed source (National Standardization Agency, 1995). Vegetative reproduction can provide seeds, such as cuttings and tissue culture techniques. To ensure that the seeds obtained are guaranteed to be uniform, testing can be carried out using PCR techniques. Vigor seeds have high growth speed, germination uniformity, growth, and good development in different environments (Sadjad, 1994).

This study showed relatively high genetic variability based on cluster analysis that formed five main groups: MO accession: Kulawi2, Kulawi10, Palolo24, Balaroa5, and Tondo19. Differences in patterns of amplified DNA bands, especially in the number and size of bands, play an essential role in determining the degree of genetic variability of plants (Fauza et al., 2007). Differences in the number and size of polymorphic and monomorphic DNA bands in gene diversity analysis largely determine the degree of diversity of a population. The high percentage of polymorphism in MO illustrates that MO in Palu Valley has a high diversity with high growth strength (vigor), including wide adaptability and resistance to environmental pressures. The increase in diversity is also due to high genetic variation. Such variations may arise because MO comes from a one-time cross between parents (Mansyah et al., 2010). Repeated hybridization among MO parents allows for wider genetic variation among parents (Sobir and Poerwanto, 2007). In addition, the variability of MO offspring can be caused by natural mutations associated with the cultivation of MO plants for thousands of years.

The study results showed that MO pollination was dominated by cross-pollination caused by the structure of flowers, namely separate male and female flowers, and supporting factors for cross-pollination, such as wind and pollinating insects in the habitat where MO is located (Alavilli et al., 2022). Pollen is distributed in an area of 20 m, indicating the presence of a pollen-mediated gene flow at a limited distance (Wu et al., 2018). Although cross-pollination dominates MO, the same genetic trait can remain dominant due to several things, such as (1) natural selection events that can increase the frequency of good alleles in the MO population, primarily when the allele is generated from self-pollination; (2) Homozygosity: In self-pollination, homozygous offspring can be produced for specific alleles, which have a high chance of being expressed in their phenotype; (3) Epistasis: An interaction between genes that causes a dominant allele inherited from one parent to mask a recessive allele inherited by another parent (Watterson, 1978; Arumingtyas, 2016). Cross-pollination can produce new alleles in the MO population so that genetic diversity and adaptability to environmental changes increase. Some essential reasons regarding the relevance of genomic analysis in the context of cross-pollination MO seed trade are: it can guarantee the genetic authenticity of seeds, producers can select seeds with superior traits that are suitable for cultivation purposes, monitor genetic diversity in seed populations so that essential genetic diversity is maintained for sustainability and how plants can adapt to environmental shifts, can identify potential inbreeding or genetic diversity low, the origin of the seeds can be accurately traced thereby increasing buyers' confidence in the products being traded, it can identify genes or alleles associated with resistance to certain diseases thereby reducing dependence on pesticides and can be used to meet the stringent regulatory

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requirements of the export or import seeds in international trade such as certification free of harmful organisms or certain diseases.

The study of 24 accessions with morphological differences showed a high proportion of genetic variation. Proven based on testing of three primers, namely $(CA)_5RG$, RY $(GACA)_4$, and $HVH(TG)_7$, it shows 100% non-identical MO accession. However, one primer $(CT)_8RG$ could not detect the DNA bands of the 24 MO accessions analyzed. This is suspected to be a mismatch between the primer and the amplified DNA.

Conclusion

Morphological identification of 150 accessions of MO Palu Valley found 24 accessions with seed viability values of 83.33 to 88.33 % and a speed of shoot emergence of 3.42 to 6.10 days. At a distance of Euclidean 0.290, five different accession groups of genotypes were found, namely accession of Kulawi2, Kulawi10, Palolo24, Balaroa5, and Tondo19, which had the potential to be used as mother trees. There is a close relationship between different MO genotype clusters and seed viability. Each MO with different genotypes has variations in seed viability and vigor, although the difference in values achieved is insignificant. This provides an opportunity to conduct further research on testing the seed vigor of each MO genotype against environmental stress.

Acknowledgements. Authors want to thank the Ministry of Education, Culture, Research, and Technology of Tadulako University for funding this research project through a prestigious research program, as stated in the Rector's Decree of Tadulako University, with reference numbers 4007/UN28/KP/2020, dated May 13, 2020, and 3654/UN28/KU/2022, dated April 11, 2022.

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